

Figures 1A-D show the identification of a complementary EPR-1 gene. A, B.

E1
Chromosomal location. A digoxigenin-labeled human P1 genomic clone selected by hybridization with the EPR-1 cDNA, was incubated with metaphase chromosomes isolated from phytohemagglutinin-stimulated PBMC in 50% formamide, 10% dextran sulfate and 2X SSC. The EPR-1-hybridizing gene was mapped in single-color labeling to the long arm of a group E chromosome (A), and in two-color staining with probe D17Z1, specific for the centromere of chromosome 17 (B), to the long arm of chromosome 17 (B), to band 17q25. C. Map of the antisense EPR-1 gene. A contig spanning 14796 bp was derived from two EPR-1-hybridizing P1 clones, subcloned in pBSKS⁺, and completely sequenced on both strands. Orientation of the map is 5'→3' with respect to the position of intron-exon boundaries (see below). Exons are solid boxes, a putative CpG island upstream exon 1 is an open box. The translational initiation codon (ATG) is indicated. Restriction sites are: B, *Bam*HI, H, *Hind*III; P, *Pst*I; S, *Sma*I; X, *Xba*I. D. Intron-exon boundaries of the antisense EPR-1 gene. Positions of the intron-exon boundaries in bp are indicated in parenthesis. The first nucleotide sequence corresponds to SEQ ID NO: 5. The second nucleotide sequence corresponds to SEQ ID NO: 6. The third nucleotide sequence corresponds to SEQ ID NO: 7.

Please replace the paragraph beginning at page 6, line 14 with the following rewritten paragraph:

E2
Figures 2A-C show the complexity and evolutionary conservation of EPR-1-related sequences. A. Southern blot of human genomic DNA. Samples were digested with the indicated restriction enzymes, transferred to GeneScreen nylon membranes and hybridized with the EPR-1 cDNA, in 5X SSC, 0.5% SDS, 5X Denhardt's and 0.1% sodium pyrophosphate at 65°C. Radioactive bands indicated by an arrow (7.6 kb *Bam*HI, 7.5 kb *Xba*I and *Hind*III fragments of 15, 7.5, 6.4, and 3.7 kb) do not derive from the antisense EPR-1 gene in Figure 1C. B. Southern blot of pulsed field gel electrophoresis. High molecular weight human genomic DNA was digested with the indicated restriction enzymes, separated by pulsed field gel electrophoresis for 20 h at 200 V with a pulse time of 75 sec, transferred to nylon membrane, and hybridized with the EPR-1 cDNA, as described in A. C. Multiple species Southern blot. *Eco*RI-digested genomic DNA from the indicated species was hybridized with a 3' 548 bp fragment of the EPR-1 cDNA, as described in A. For all panels, molecular weight markers in kb are shown on the left.

Please replace the paragraph beginning at page 6, line 28 with the following rewritten paragraph:

E3
Figures 3A-F show the discordant tissue distribution of sense/antisense EPR-1 transcripts. Northern hybridization was carried out on a multiple tissue adult (A-C) or fetal (D-F) mRNA blot with single strand-specific probes in 5X SSPE, 10X Denhardt's solution, 2% SDS, 100 mg/ml denatured salmon sperm DNA at 60°C for 14 h. After washes in 2X SSC at 60°C and in 0.2X SSC at 22°C, radioactive bands were visualized by autoradiography. A and B. EPR-1-specific single-strand probe. D and E. Antisense EPR-1-specific single-strand probe. C and F. Control actin probe. Molecular weight markers in kb are shown on the left.

Please replace the paragraph beginning at page 7, line 6 with the following rewritten paragraph:

E4
Figures 4A-C show the sequence analysis of Survivin and expression in cell lines. A. Predicted translation of the antisense EPR-1 gene product (Survivin)(SEQ ID NO: 34). B. Sequence alignment of the BIR in Survivin (SEQ ID NOS: 8 and 21) and in other IAP proteins by the Clustal method. IAP proteins are identified by accession number, L49433 (SEQ ID NOS: 9 and 22), TNFR2-TRAF signaling complex-associated IAP; L49441 (SEQ ID NOS: 10 and 23), apoptosis 2 inhibitor (Drosophila); P41436 (SEQ ID NOS: 11 and 24), IAP gene from Cydia pomonella granulosus virus; P41437 (SEQ ID NOS 12 and 25), IAP gene from Orgyia pseudotsugata nuclear polyhedrosis virus; U19251 (SEQ ID NOS: 13 and 26), NAIP, neuronal inhibitor of apoptosis; U32373 (SEQ ID NOS 14 and 27), IAP-like protein ILP from Drosophila melanogaster; U32974 (SEQ ID NOS: 15 and 28), human IAP-like protein ILP; U36842 (SEQ ID NOS: 16 and 29), mouse inhibitor of apoptosis; U45878 (SEQ ID NOS: 17 and 30), human inhibitor of apoptosis 1; U45879 (SEQ ID NOS: 18 and 31), human inhibitor of apoptosis 2; U45880 (SEQ ID NOS: 19 and 32), X-linked inhibitor of apoptosis; U45881 (SEQ ID NOS: 20 and 33), Drosophila inhibitor of apoptosis. Conserved residues are boxed, identities between Survivin and NAIP (U19251) ; SEQ ID NOS: 13 and 26 are boxed and shaded. C. Immunoblotting with anti-Survivin antibody JC700. Protein-normalized aliquots of SDS-extracts of cell lines HEL (erythroleukemia), Daudi and JY (B lymphoma), THP-1 (monocytic), Jurkat and MOLT13 (T leukemia), or non transformed human lung Lu18 fibroblasts, HUVEC or

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E4
PBMC were separated by electrophoresis on a 5-20% SDS gradient gel, transferred to Immobilon and immunoblotted with control non-immune rabbit IgG (RbIgG), or anti-Survivin antibody JC700 (Survivin). Protein bands were visualized by alkaline phosphatase-conjugated goat anti-rabbit IgG and tetrazolium salts. Molecular weight markers in kDa are shown on the left.

Please replace the paragraph beginning at page 7, line 26 with the following rewritten paragraph:

E5
Figures 5A-C show the regulation of Survivin expression by cell growth/differentiation. HL-60 cells were terminally differentiated to a mature monocytic phenotype by a 72 h culture with 0.1 mM vitamin D₃ plus 17.8 mg/ml indomethacin. Survivin expression before or after vitamin D₃ differentiation was detected by immunoblotting with JC700 antibody, or by Northern hybridization with a Survivin-specific single strand probe. RbIgG, control non-immune rabbit IgG. Protein molecular weight markers in kDa and position of ribosomal bands are shown on the left of each blot.

Please replace the paragraph beginning at page 8, line 4 with the following rewritten paragraph:

E6
Figures 6A-H show the over-expression of Survivin in human cancer, *in vivo*. A. Immunohistochemical staining of human lung adenocarcinoma with affinity-purified anti-Survivin antibody JC700 (20 µg/ml). B. Inhibition of JC700 staining of lung adenocarcinoma by pre-absorption with the immunizing Survivin 3-19 peptide. C. Immunohistochemical expression of Survivin in squamous lung cell carcinoma, but not in the adjacent normal gland epithelium of the lung (C, arrow). D. *In-situ* hybridization of Survivin mRNA in squamous lung cell carcinoma with a Survivin-specific riboprobe. E. Expression of Survivin in pancreatic adenocarcinoma by immunohistochemistry with JC700. F. Normal pancreas, negative for Survivin expression by immunohistochemistry. G. *In situ* hybridization of Survivin mRNA expression in colon adenocarcinoma, but H, not in the adjacent non neoplastic colon gland epithelium (H, arrow). Magnifications are x200, except G, x400.

Please replace the paragraph beginning at page 8, line 16 with the following rewritten paragraph:

E7 Figures 7A-C show the effect of Survivin on apoptosis/proliferation. A. EPR-1-regulation of Survivin expression. HeLa cells were transfected with control vector pML1 or the EPR-1 cDNA (which is antisense to Survivin) by electroporation, and selected in hygromycin (0.4 mg/ml). Aliquots of vector control HeLa cells (Vector) or Survivin antisense transfectants (Antisense) were induced with 200 mM ZnSO₄ detergent-solubilized, and immunoblotted with the anti-Survivin JC700 antibody. Molecular weight markers are shown on the left. B. Effect of Survivin on apoptosis. Survivin antisense transfectants (1, 2), or vector control HeLa cells (3, 4) were induced with Zn²⁺ ions in 0% FBS for 24 h and stained by the AptoTag method with TdT-catalyzed dUTP labeling of 3'-OH DNA ends and immunoperoxidase (1, 3), or by hematoxylin-eosin (HE) (2, 4). 1. Prominent nuclear DNA fragmentation detected by AptoTag staining in serum-starved Survivin antisense transfectants; 2. HE staining of antisense transfectants reveals the presence of numerous apoptotic bodies (arrows); 3. AptoTag staining of vector control HeLa cells detects a few sparse apoptotic cell (arrow); 4. HE staining of vector control HeLa cells. The arrow indicates a single apoptotic body. Magnification x400. C. Effect of Survivin on cell growth. Twenty thousands vector control HeLa cells (Vector) or Survivin antisense transfectants (Antisense) were seeded in 24-well plates, induced with ZnSO₄, harvested at the indicated time points, and cell proliferation was determined microscopically by direct cell count. Data are the mean ± SEM of replicates of a representative experiment out of seven independent determinations.

Please replace the paragraph beginning at page 9, line 7 with the following rewritten paragraph:

E8 Figures 8A-D show the expression of Survivin in HL-60 cells. HL-60 cells were examined via Western and Northern blots for Survivin expression.

Please replace the paragraph beginning at page 9, line 12 with the following rewritten paragraph:

E9 Figures 10A-G show the nucleotide Sequence of *Survivin*, which corresponds to SEQ ID NO: 35. The amino acid sequence displayed in Figure 10 corresponds to SEQ ID NO: 34.

Please replace the paragraph beginning at page 9, line 13 with the following rewritten paragraph:

E10 Figures 11A-C show the expression of Survivin and the generation and characterization of anti-Survivin mAb 8E2 by ELISA and immunoblotting.

Please replace the paragraph beginning at page 10, line 3 with the following rewritten paragraph:

E11 Figures 14A-B show that the presence of Survivin is a negative predictive-prognostic factor in neuroblastoma.

Please replace the paragraph beginning at page 10, line 5 with the following rewritten paragraph:

E12 Figures 15A-B show that the presence of Survivin is a negative predictive prognostic factor in high-grade non-Hodgkin's lymphoma.

Please replace the paragraph beginning at page 22, line 7 with the following rewritten paragraph:

E13 As used herein, "stringent conditions" are conditions in which hybridization yields a clear and detectable sequence. Stringent conditions are those that (1) employ low ionic strength and high temperature for washing, for example, 0.015 M NaCl, 0.0015 M sodium citrate, 0.1% SDS at 50°C; or (2) employ during hybridization a denaturing agent such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42°C. Another example is use of 50% formamide, 5x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2x SSC and 0.1% SDS. A skilled artisan can readily determine and vary the stringency conditions appropriately to obtain a clear and detectable hybridization signal.